Lasers in Medical Science (2025) 40:153
https://doi.org/10.1007/s10103-025-04410-1

RESEARCH

Effects of photobiomodulation on adipocytic infiltration in sites of skin healing: in vivo experimental study

Beatriz Paim de Figueiredo Braitenbach[1] · Carla Barreto Silva de Cerqueira[1] · Sarah Souza Lima[1] · Isadora de Carvalho Hegouet[1] · Bruna Carvalho Lopez Moreno[2] · Alena Ribeiro Alves Peixoto Medrado[1]

Received: 18 September 2024 / Accepted: 12 March 2025

Abstract

Adipocyte infiltration consists of a temporary increase in the number of adipocytes in the microenvironment of tissue injury. There is already evidence in the literature of adipocytes' participation in promoting the inflammatory response, and parallelly laser photobiomodulation can benefit the tissue repair process. This study aimed to chronologically analyze adipocytic infiltration in the repair of photobiomodulated skin wounds experimentally induced in rats through histomorphometric analysis. The sample consisted of 20 rats divided into 2 groups: control group and group subjected to laser photobiomodulation. The skin portions of the back of rats were processed and stained with Hematoxylin-Eosin in 4 µm thick sections including the surgical wound 5 and 10 days after the proposed treatments. Qualitative and quantitative analyses were performed by capturing images of tissue sections, describing the organizational pattern of adipocytes around the surgical wound and counting individual adipocytes in the connective tissue in formation. Adipocytic infiltration was observed in both experimental groups on the 5th day, with a decrease on the 10th day. The group treated with photobiomodulation presented a greater number of adipocytes compared to the control group, in both periods analyzed. The findings of the present study seem to corroborate the literature, which indicates that adipose cells might stimulate inflammation and repair, and photobiomodulation can enhance these effects, since it aids the process of adipocytic infiltration in the injured area. Clinical trial number: Not applicable.

Keywords Adipocyte infiltration · Adipocytes · Repair · Inflammation · Laser photobiomodulation

Introduction

The occurrence of chronic wounds is an important issue for the health sector, as their incidence has been increasing [1–3]. Such injuries present an interrupted healing process, remaining unhealed for, in general, a period of three months or more [4]. Therefore, they will require longer hospitalization time and financial resources for their treatment, and may also compromise the recovery of patients. Such injuries require longer hospitalizations and financial resources for their treatment and may compromise patients' recovery. Old age and the presence of comorbidities such as diabetes mellitus, systemic arterial hypertension and obesity are some factors that are associated with difficulty in healing [5, 6].

Cellular injuries caused by physical trauma, oxygen deprivation, chemical and infectious agents are events that trigger inflammation and the repair process, which culminate in an attempt to restore the tissue's structure and function. Aggressions of any nature may determine the

✉ Alena Ribeiro Alves Peixoto Medrado
armedrado@ufba.br

Beatriz Paim de Figueiredo Braitenbach
beatrizpaimfb@gmail.com

Carla Barreto Silva de Cerqueira
carlacerqueira@ufba.br

Sarah Souza Lima
sarah.lima@ufba.br

Isadora de Carvalho Hegouet
isadorahegouet@gmail.com

Bruna Carvalho Lopez Moreno
brunamoreno20.1@bahiana.edu.br

[1] Federal University of Bahia, Salvador, Brazil

[2] Bahiana School of Medicine and Public Health, Salvador, Brazil

Published online: 20 March 2025

occurrence of a series of reactions in the body, which aim at the morphofunctional repair of the affected tissue. The hemostasis process, which takes place immediately after injury, limits blood loss and promotes platelet activation, followed by the inflammatory response with the recruitment of cells that phagocytose pathogens and release a series of chemical mediators, such as cytokines, prostaglandins and reactive oxygen species. In the proliferative phase of tissue repair, successive mitoses can be observed involving keratinocytes, adipocytes, endothelial cells and fibroblasts, as well as parenchymal cells originally present in the tissue. There is also a rise in the biosynthesis of structural components of the extracellular matrix. Finally, synthesis and degradation of the new extracellular matrix deposited at the site of injury takes place simultaneously in the repair phase known as remodeling [7, 8].

A growing number of studies have investigated a phenomenon known as adipocytic infiltration that occurs in the microenvironment of tissue injury. This is a temporary increase in adipocytes in the connective tissue corresponding to the area of injury, which precedes the granulation tissue and extends until the end of the proliferative phase. There is already evidence in the literature of the adipocytes participating in promoting the inflammatory response, both in vitro and in vivo [9–11]. It is known that adipose cells, besides storing nutrients to provide energy in times of metabolic need, also have an endocrine function which consists in releasing cytokines and growth factors, and thus, can directly influence the activity of immune system cells. Regarding dermal adipocytes, their ability to regulate local inflammation and their prominent role in recruiting macrophages to the injured area have also been analyzed. These macrophages are able of detecting injury patterns through Toll-like receptors (TLRs), which activate pro-inflammatory nuclear factor kappa B pathway and stimulate the biosynthesis of several chemokines, such as CCL3, CXCL10, intercellular adhesion molecule 1, and the cytokines IL-6, IL-8, and TNF-α, which promote inflammation [8]. These chemical mediators can stimulate additional migration of adipocytes from the hypodermis and the transdifferentiation of adult stem cells present in the dermis.

In the study by Schmidt & Horsley [12], the authors analyzed the adipocytes activation during the healing of cutaneous wounds and reported that the percentage of their precursors increased in the injured region compared to uninjured skin, after 5 and 7 days. It was also analyzed that the inhibition of adipocyte transdifferentiation during the repair process also consequently inhibited the recruitment of fibroblasts to the site of the injury. This finding justified the occurrence of defective remodeling that can compromise the integrity of the scar, specifically during late healing. In addition, the study data showed that proliferative phase of the repair involved repopulation of adipose cells in the extension of the tissue which suffered the injury, through migration and adipogenesis. The authors suggest that the activation of pre-adipocytes may concomitantly occur with the infiltration of immune system cells into the wound bed.

The properties of adipose tissue have been increasingly studied as measures to be applied in clinical practices. Stem cells derived from adipose tissue were isolated in 2001 by Zuk, Zhu and Mizuno [13], and their potential for differentiation into osteoblasts, chondroblasts, adipocytes, vascular endothelial cells and myocytes was described. Tissue engineering studies have demonstrated that such cells have shown promise for regenerative therapy due to their easiness of collection, their availability in the human body and the feasibility of their in vitro culture [14].

The literature has shown that low-level laser therapy, also known as laser photobiomodulation, can benefit the healing process. The authors have previously investigated the influence of photobiomodulation on tissue repair in rats and demonstrated that the group submitted to treatment presented a reduction in the wound area and exudation in the initial phase, a better organization of collagen fibers during remodeling and a series of morphological changes favorable to repair, even though the healing time was not reduced in relation to the control group [15]. They also observed the presence of adipocytic infiltration on the wounds' bed, although this finding was not described in the study. This morphological finding awakened their attention about the initial hypothesis whether photobiomodulation could interfere in the amount of these cells.

Since laser photobiomodulation has been widely used as an adjuvant therapy for the management of wounds with difficult healing, such as oral mucositis, pressure ulcers, venous ulcers and infection, among other clinical conditions [16–19], it would be important to investigate whether the process of adipocytic infiltration could be influenced by photobiomodulation. And also, if the use of laser would be able to contribute to increasing the presence of adipocytes in the wound bed. In order to answer this question, the authors carried out the present morphological histomorphometric study.

Materials and methods

Study design

Prospective and experimental study, registered under protocol number 67/2019 and approved by the Ethics Committee on the Use of Animals (CEUA) of the Faculdade Adventista da Bahia (FADBA), with approval report number 01.0039.2013.

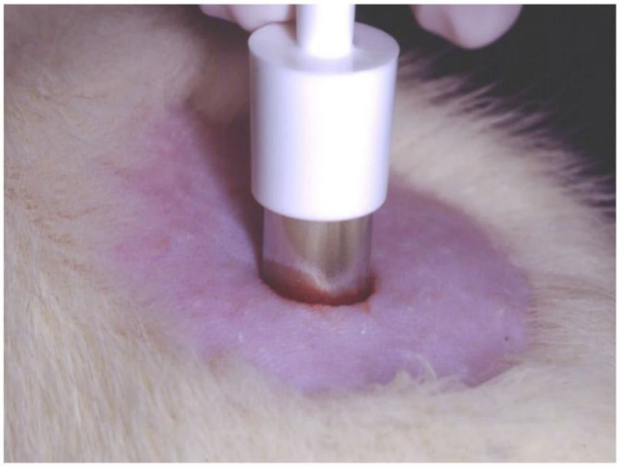

Fig. 1 Circular incision made in the dorsal region using a circular metal punch scalpel.
Source: Authors

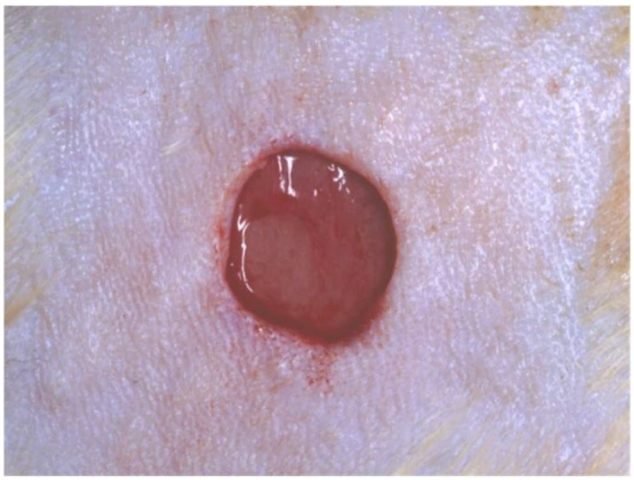

Fig. 2 Clinical aspect of the circular wound.
Source: Authors

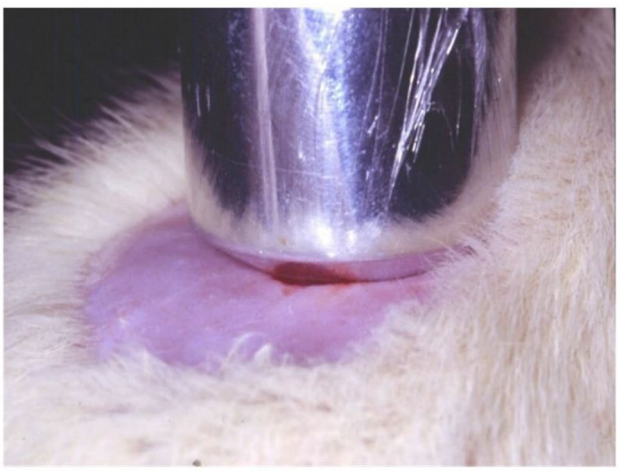

Fig. 3 Application of laser photobiomodulation.
Source: Authors

Study samples

The protocol of this research has already been published in the literature [20]. The sample included 20 male Wistar rats weighing between 200 and 300 g, with an average age of 8 weeks, corresponding to young adult animals, from the vivarium of Faculdade Adventista da Bahia. by drawing lots, rats were randomly divided into 2 groups of 10 rats each, which were killed 5 and 10 days after the proposed treatments. Group 1 corresponded to the Control Group (CG) and did not receive any treatment, except contact with the active tip of the switched-off laser; Group 2, named the Laser Group (LG) was submitted to conventional laser photobiomodulation by contact, with light emission.

Surgical procedures

The animals were weighed and anesthetized with 10% ketamine hydrochloride (Dopalen®, São Paulo, Brazil) 75 mg/mL and 2% xylazine hydrochloride (Anazedan, São Paulo, Brazil) 5 mg/mL, at doses of 2 mg/kg and 3 mg/kg, respectively. The dorsal trichotomy and antisepsis with povidone-iodine (Rioquímica, São Paulo, Brazil) were then performed.

A circular incision was made in the dorsal region using a circular metal punch scalpel (Biopsy Punch, Stiefel, Germany) with a diameter of 6 mm to obtain a uniform and standardized wound, which was performed by a single, properly calibrated operator according to the model described by Medrado et al., in 2010 [21], (Figs. 1 and 2).

The animals were immobilized during the laser application by two researchers properly trained for this purpose, who held the cranial and caudal portions, respectively, with the aid of a soft tissue. The skin wounds of the rats in the LG were submitted to 4 punctual applications of 1 J/cm², during 32 s, with a total dosimetry of 4 J/cm² per day of application (Fig. 3). A semiconductor aluminum gallium arsenide laser device (AsAlGa, 9 mW, 670 nm, 0.031 W/cm^2 diode laser) was used, with continuous emission and an active tip area of 0.28 cm² (Laser VR-KC-610-Dentoflex, Brazil). Regarding dosimetry, photobiomodulation was applied at 4 points corresponding to the diametrical vertices of the circular wound. The dose used at each point was 1 J/cm^2. Thus, the animals received a total of 4 J/cm^2 each day, for 3 days, with a total dose of 12 J/cm^2 since the rats were irradiated for 3 consecutive days after the surgical procedure. The animals underwent the biomodulatory therapy described on days 1, 2 and 3 of the study. The CG underwent the same procedure with the device turned off.

Death of animals

Each group of 10 animals had half of the specimens sacrificed on the 5th day and the other half on the 10th day. After deep sedation with the anesthetic solution already described, animals were placed in boxes in which carbon dioxide was released at a concentration of 5 L per minute.

Histological processing

After confirmation of rats' death, a portion of skin was removed from the back, including the surgical wound. The material was fixed for a period of 48 h in a buffered 10% formalin solution. After, this tissue was processed for staining with Hematoxylin-Eosin in 4 µm thick sections.

Histomorphometry

The histomorphometric evaluation was performed according to the study protocol published by Lima et al. [20]. The images of the tissue sections subjected to the described staining were captured using the Motic Images Advanced 3.0 ® software (Motic China Group CO. LTD) at the Oral Biochemistry Laboratory of the Institute of Health Sciences of the Federal University of Bahia. A standard area for analysis of all cases (100 μm^2) was established, which comprised the central region of the wound with the adjacent normal tissue as lateral limits. Three standard images, which approached the two edges of the lesion (right and left) and the center corresponding to each case, were captured with the pre-established dimension and used for both qualitative and quantitative analysis of individual adipocytes. Thus, it was possible to map the entire wound region and to identify the transition between the intact skin and the wound, in order to verify from which region, the adipocytes could have migrated. Each area was captured at 400x magnification and saved in JPEG format. All analyses were performed and documented by a single calibrated and highly experienced examiner.

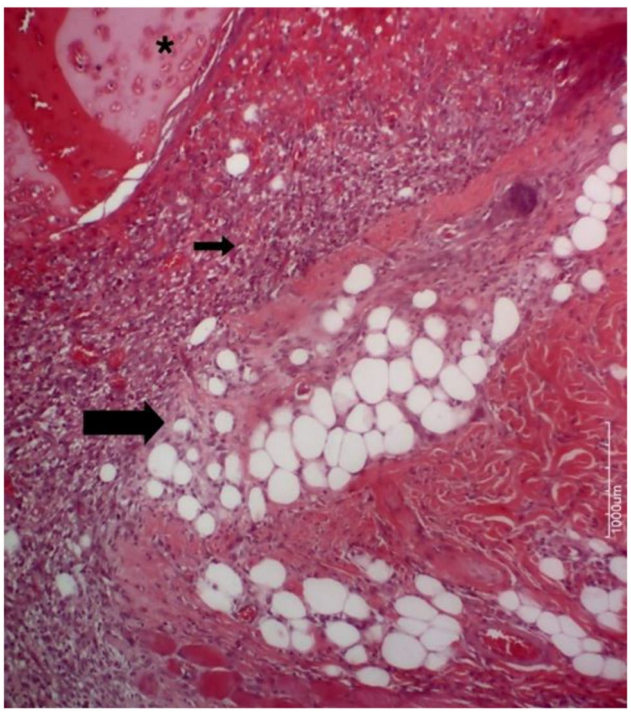

Fig. 4 Adipocyte infiltration represented by numerous vacuolated cells amidst the granulation tissue of the lesion's derm on the 5th day in the CG. In this figure, it is possible to identify the wound crust (asterisk), the granulation tissue (smaller arrow) and the infiltration of cells with adipocytic phenotype (larger arrow). Hematoxylin-eosin, 400X. Source: Authors

Statistical analysis

The data were analyzed using R software (version 4.3.2). Descriptive statistics were performed, establishing means, standard deviations, medians and their respective interquartile ranges, according to the two independent intervention groups. Based on the characteristics of the sample size and the variables chosen for analysis, non-parametric tests ANOVA, Student's t-test and Fisher's exact test were used to identify the general and specific characteristics of the data. Fischer's exact test was used to verify a significant association between variables. The significance level established for this study is 5%.

Results

At 5 days, in the sections stained with hematoxylin-eosin, it was noticed in both experimental groups adipocytic infiltration, represented by rounded cells with vacuolated cytoplasm and eccentric nuclei which were distributed in islands and sheets in the dermis corresponding to the site of the lesion (Figs. 4 and 5). These cells with adipocytic phenotype were infiltrating the area corresponding to the granulation tissue. In this proliferative phase of the repair, it was possible to observe the advanced formation of the typical granulation tissue, consisting of numerous newly formed blood vessels and intense fibroblast proliferation with sparsely distributed monomorphonuclear inflammatory cells. On the 10th day, both groups exhibited increasing fibroplasia in the lesion area, represented by dense collagen bundles, with a clear decrease in the population of cells with adipocyte phenotype ($p > 0{,}05$) (Figs. 6 and 7).

In the CG, a median of 114 adipocytes was observed on the 5th day of the postoperative period and 55 on the 10th day, with an evident decrease in these cells amount ($p > 0{,}05$); (Table 1). In the experimental group submitted to

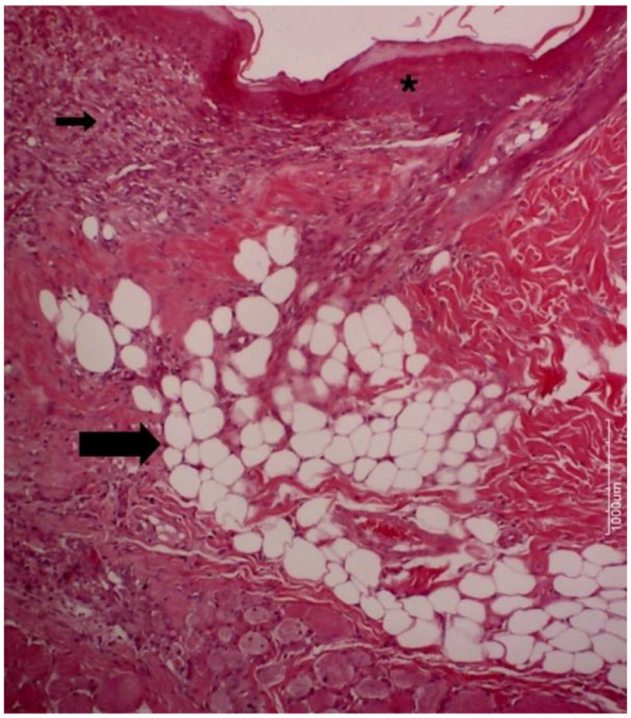

Fig. 5 Adipocyte infiltration represented by numerous vacuolated cells amidst the granulation tissue of the lesion's derm on the 5th day in the LG. In this figure, it is possible to visualize crust with evident reepithelialization below (asterisk), granulation tissue (smaller arrow) and infiltration of cells with adipocytic phenotype (larger arrow) in the dermis. Hematoxylin-eosin, 400X.
Source: Authors

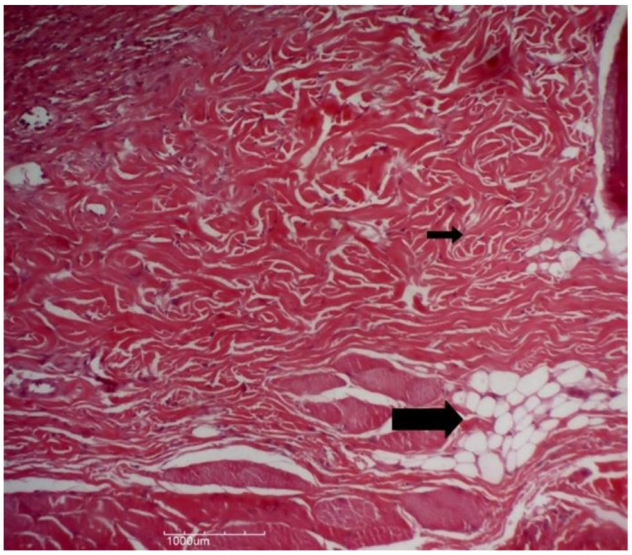

Fig. 6 Adipocyte infiltration represented by numerous vacuolated cells in the extracellular matrix of the lesion on the 10th day, in the CG. In this figure, it is possible to observe fibroplasia of the dermis (smaller arrow) and the infiltration of cells with adipocytic phenotype (larger arrow) on one of the lateral edges of the lesion. Hematoxylin-eosin, 400X.
Source: Authors

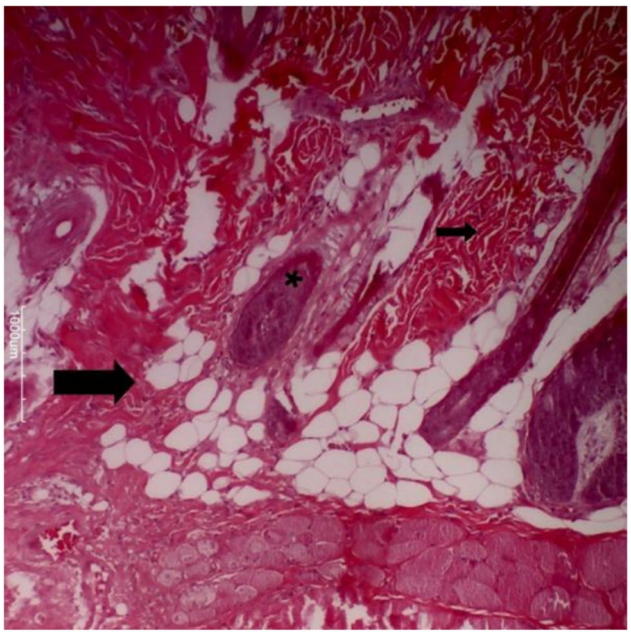

Fig. 7 Adipocyte infiltration represented by numerous vacuolated cells in the extracellular matrix of the lesion on the 10th day, in the LG. In this figure, it is possible to recognize skin appendages (asterisk), area of fibroplasia (smaller arrow) and infiltration of cells with adipocytic phenotype (larger arrow) in the dermis. Hematoxylin-eosin, 400X.
Source: Authors

Table 1 Medians and interquartile ranges of adipocyte counts as a function of experimental group and time, intragroup analysis, Hematoxylin-eosin, 400X

Group	5 days	10 days	*p*-value*
CG	114 (63,5–132,5)	55 (24–65,5)	0,14
LG	170 (74–300,5)	95 (79,5–191,5)	0,11

* Fischer test. CG represents control group e LG laser group. Source: Own authorship

laser photobiomodulation, the median number of adipocytes corresponding to the 5th day was 170 with a decrease on the 10th day, and the median value was 95 cells in the evaluated area ($p > 0,05$) (Table 1).

In the intergroup analysis, the LG showed a higher number of cells with an adipocytic phenotype in relation to the CG, both on the 5th and 10th days of the histomorphometric analysis from the skin sections, and such difference was statistically significant ($p = 0,03$; $p = 0,04$, respectively); (Table 2).

Discussion

The present investigation aimed to comparatively evaluate the process of adipocytic infiltration in the bed of experimentally induced skin wounds in rats through histomorphometric analysis. One of the experimental groups was treated with laser photobiomodulation. Experimental studies have

Table 2 Medians and interquartile ranges of adipocyte counts as a function of experimental group and time, intergroup analysis, Hematoxylin-eosin, 400X

Time	CG	LG	*p*-value*
5 days	114 (63,5–132,5)	170 (74–300,5)	0,03
10 days	55 (24–65,5)	95 (79,5–191,5)	0,04

* Fischer test. CG represents control group e LG laser group. Source: Own authorship

investigated the effects of low-intensity laser application on ulcer healing in recent decades, showing it to be a promising therapy both in vitro and in vivo [15, 22–24]. It is known that the therapeutic administration of light energy can modulate cellular activity in a living biological system, since the light is absorbed by molecular photoreceptors, and can influence cellular functions including the process of tissue regeneration [25]. However, although adipocytic infiltration has been descriptively documented, up to date this study is the first to evaluate the number of cells with an adipocytic phenotype in the proliferative phase of tissue repair of photobiomodulated wounds in vivo.

In mammals, there are two types of adipose tissue that can be histologically differentiated and have antagonistic functions, the white adipose tissue (WAT) and the brown adipose tissue (BAT). Adipocytes constitute adipose tissue along with other cell types and are the only cells that have the ability to store lipids without compromising their functional integrity. They are able to synthesize fatty acids, store excess calories in the form of triacylglycerol and break them down, via lipolysis, into free fatty acids and glycerol, when there is an energy need [26].

Brown adipocytes found in greater quantity in human adipose tissue in the early phase of life, are cells specialized in thermogenesis through lipid oxidation, actively participating in body temperature regulation. Its morphology is characterized by multiple lipid vacuoles in its cytoplasm, an increased number of mitochondria and expression of uncoupling protein 1 (UCP-1), which acts as a proton channel in the inner mitochondrial membrane, diverting these protons from ATP synthesis and allowing potential energy to be dissipated in the form of heat. On the other hand, the adipocytes that make up the WAT are large cells that change in size depending on the number of triglycerides they store in a unilocular lipid vacuole, which can occupy 85 to 95% of its cytoplasm. WAT is the predominant type of adipose tissue in adults, and although it is mainly concentrated in the subcutaneous region and visceral organs, it is distributed in a generalized manner throughout the body. Among its functions, in addition to energy storage, it is possible to highlight mechanical protection against shocks and traumas and maintenance of body temperature [9, 27].

In addition to producing factors that operate the body's energy balance and thermoregulation, it has been shown that adipose tissue cells also secrete cytokines, growth factors and pro-inflammatory factors, called adipokines, which influence other different metabolic processes including inflammation and repair [28]. Thus, adipose tissue may even be placed side by side with traditional endocrine organs such as the pancreas, ovaries, testicles and adrenal gland, since it is important for general metabolic homeostasis [9]. In the present study, adipocytic infiltration was observed on both the 5th and 10th day of analysis, in both experimental groups. During these periods, proliferative processes begin to predominate in the microenvironment of the lesion and the presence of adipocytes in the dermis seems to indicate a temporary participation of these cells in tissue repair.

It has been reported in the literature that the action of adipocytes on healing takes place through the modulation of macrophages and fibroblasts recruitment, in addition to stimulating the biosynthesis of inflammatory mediators. Human omental adipocytes express the chemokines CCL2 and CXCL8, while subcutaneous adipocytes produce adiponectin which mobilizes the anti-inflammatory polarization of macrophages, CCL3, CCL5, CXCL1, CXCL5 and leptin that stimulates pro-inflammatory responses in macrophages and neutrophils [10]. Through Toll-like receptors, adipocytes might detect and respond to inflammatory stimuli, such as lipopolysaccharides, through the pro-inflammatory nuclear factor kappa B (NF-κB) pathway, which results in the production of CCL3, CXCL10, intercellular adhesion molecule 1 (ICAM1), IL-6, IL-8/CXCL8, and TNF-α. Adipocytes also respond to TNFα and IL1 ligands and are involved in neutrophils and macrophages recruitment [8].

It is important to emphasize that there are established differences between subcutaneous adipose tissue and dermal white adipose tissue (DWAT), which in rodents and humans is found as a superficial layer above the subcutaneous adipose tissue, also known as hypodermis [29]. Driskell et al. [30] proposed this denomination after observing that the origin of this adipose deposit below the reticular dermis is independent of the origin of the subcutaneous adipose tissue, and many authors still referred to both as if they were the same. DWAT, in addition to having its stores of triglycerides enriched with lipids capable of regulating inflammation, expresses CCl4 and secretes antimicrobial peptides, reinforcing its role in host defense [8]. Zhang et al. [31] also investigated DWAT and reported that it demonstrates high plasticity and stands out among other fat deposits due to its great capacity to expand, in number and size, and contract in response to various stimuli such as different phases of the hair follicle cycle, high-fat diets and local infections. The mechanisms of these cells in wound healing and host defense continue to be studied, since their biological task has not yet been fully understood.

Regarding the proliferative phase of tissue repair, the study by Schmidt & Horsley [12] demonstrated the repopulation of adipose cells in the extension of the injured tissue, through the analysis of migration and inhibition of adipogenesis with peroxisome proliferator receptor gamma (PPARγ) inhibitors. It has been suggested that preadipocyte activation may concomitantly occur with the infiltration of immune system cells into the wound bed. Furthermore, by observing the reduction in the number of fibroblasts in mice with inhibited adipogenesis, it was possible to state that adipocytes contribute to the recruitment of these cells into skin wounds and that there is direct intercellular communication between both. Like platelets, mature adipocytes, produce platelet-derived growth factor (PDGF) ligands in the skin, which among its functions, acts as a chemotactic agent for neutrophils, macrophages and fibroblasts, contributing to their migration during dermal healing of skin wounds. Furthermore, the authors identified that dermal defects that occur in the absence of adipocytes led to defects in dermal remodeling which compromised the integrity of closed wounds, resulting in significantly larger wound bed areas and recurrence.

The adipocytic infiltration in the repair phase corresponding to granulation tissue suggests that this cellular modulation results from the phenotype changing of adult mesenchymal stem cells or fibroblasts differentiated into adipocytes and may be very relevant for the proliferative and remodeling phase of repair [32, 33]. In the present study it was observed that in sections of normal rat skin, fat cells are usually organized in the adipose tissue represented by the hypodermis, but not in the wound area. Regarding inflammatory phase, adipocytic infiltration was not present, due to the accumulation of exudate in the interstitium. During remodeling phase, the increasing concentration of collagen in the wound area did not demonstrate the presence of adipocytes too. However, in the proliferative phase of healing, it was possible to see elongated cells with fat cytoplasmic inclusions that also exhibited an increase in the rough endoplasmic reticulum. It seemed to be a temporary change in phenotype.

We do not know whether this phenotypic modulation may come from gene reprogramming of fibroblasts, pericytes, mesenchymal stem cells or even macrophages. All these cells can transitionally change their phenotype; however, it is not clear the function of this cellular modulation. As a matter of fact, in a previous study of Medrado et al. [34], these authors used the same experimental model and the ultrastructural examinations showed the presence of elongated cells with fat cytoplasmic inclusions, which were identified as fibroblasts. These cells present in the extracellular matrix exhibited an increase in the rough endoplasmic reticulum, which indicated their intense protein synthesis activity. According to these findings from our previous study, the authors believe that the cellular debris released as a result of the initial inflammatory reaction could be endocytosed by fibroblasts and/or myofibroblasts present in the wound area.

It has also been described that adipocytes also play an important role in the fibrosis process. Stem cells derived from adipose tissue have a regenerative and antifibrotic effect, since they are capable of inhibiting TGF-β1, one of the main molecules involved in the synthesis of ECM, altering the balance between its production and degradation and thus facilitating the decomposition of fibrotic tissue [35]. In addition to fibroblasts, in the wound contraction phase other cell types such as epithelial and endothelial cells, macrophages and adipose cells can transition to myofibroblasts in the skin. Cells expressing adiponectin achieve expression of α-actin 2 (ACTA2) and lose the adipogenic gene, which demonstrate the transdifferentiation of adipocytes into myofibroblasts [33]. In previous studies by our research group, phenotypic differentiation of adipocytes in photobiomodulated skin wounds was frequently observed in histological sections, although it was never the exclusive object of analysis [36, 37]. Therefore, the present study confirmed these findings and focused on performing histomorphometric analysis that allowed counting the number of cells with adipocyte phenotype in the injured dermis. When control and photobiomodulated groups were compared at different periods of skin repair, photobiomodulation was able to significantly stimulate adipocyte infiltration in the injured area.

Although it was not the matter of this study, the authors hypothesized that such findings could be related to the laser's ability to promote lipolysis of adipocytes present in the hypodermis, whose main products are palmitoleic acid, oleic acid, α-linoleic acid, and medium-chain fatty acids. It is possible that such lipolysis process in the dermis is related to the recruitment of fibroblasts and/or monocytes and their differentiation into adipocytes, since they express multiple receptors and fatty acid transporters during the chronic inflammatory phase. And might indirectly promote stimuli for additional fibroblast proliferation, cellular transdifferentiation, and faster healing resolution [38].

In this sense, Pourhashemi et al. [39] evaluated the effects of photobiomodulation and injection of stem cells derived from human adipose tissue on the inflammatory and proliferative phases of infected, ischemic and impaired healing wounds in rats with type 1 diabetes mellitus. Laser and stem cells administration together demonstrated better results than both therapies alone, in relation to wound strength, reduction of colony-forming units, reduction of the inflammatory reaction and rise in proliferative activity, expressed by a higher fibroblast count and formation of new blood vessels, when compared to the control group.

The secretome of these cells can also play vital roles in efficient healing, such as altering the phenotype of macrophages during the inflammatory phase, promoting angiogenesis, increasing cell migration and differentiation of endothelial cells for the formation of new vessels, facilitating granulation tissue and extracellular matrix production [40]. Photobiomodulation can enhance these effects by stimulating adipocytic infiltration in the injured area by increasing the expression of angiogenic factors, viability and migration of these cells [41].

The main limitation of this study is to be focused on the analysis of a single variable: adipocytic infiltration. Hematoxylin-Eosin staining might not effectively differentiate fat cells from various sources, but it allowed the description of tissue morphology that was infiltrated by adipocytes. Also, the results presented by this study do not allow us to identify the possible origin of adipocytic infiltration. However, it represents a starting point for future studies correlating other variables, such as the number of fibroblasts and myofibroblasts, vascular density and area of collagen biosynthesis. In the same way, these results may support studies that focus on comparing the use of other biomodulator therapies in healing and their influence on adipocytes or analyzing tissue repair in other biological contexts, for example wounds infected by microorganisms or hypoxic.

Conclusion

In the proliferative phase of skin tissue repair, a higher number of adipocytes was observed on the 5th day, with a decrease on the 10th day postoperatively, in the area corresponding to the granulation tissue. In the experimental group subjected to laser photobiomodulation, the number of adipocytes was significantly higher which seems to indicate a greater mobilization of adipose cells that will act as physiological pool to support skin regeneration and repair. For future studies, the authors encourage the development of new investigations that include immunohistochemistry and/or molecular biology techniques to determine the possible origin of the adipocytic infiltration.

Acknowledgements The authors thank Bahia State Research Support Foundation (FAPESB) and Coordination for higher Education Staff Development of Brazil (CAPES) for supporting this research.

Author contributions All authors contributed to the study conception and design. Material preparation was performed by Alena Ribeiro Alves Peixoto Medrado, while analysis and data collection were executed by Beatriz Paim de Figueiredo Braitenbach and Alena Ribeiro Alves Peixoto Medrado. The first draft of the manuscript was written by Beatriz Paim de Figueiredo Braitenbach and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding The research leading to these results received funding from the Bahia State Research Support Foundation (FAPESB)– Brazil, under application number 3702/2023.

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

References

1. Menezes SM, Fonseca AKB, Matos NM (2022) Perfil de Pacientes com lesões Cutâneas hospitalizados Em Uma Unidade de Internação de clínica médica. Health Residencies J 3:95–108. https://doi.org/10.51723/hrj.v3i15.426
2. Mittag BF, Krause TCC, Roehrs H, Meier MJ, Danski MTR (2017) Cuidados com Lesão de Pele: Ações Da enfermagem. ESTIMA [Internet] 15(1). https://doi.org/10.5327/Z1806-3144201700010004
3. Resende NM, Nascimento TC, Lopes FRF, Júnior AGP, Souza NM (2017) Cuidado de Pessoas com feridas crônicas Na Atenção primária à Saúde. J Manag Prim Health Care [Internet] 8(1):99–108. https://doi.org/10.14295/jmphc.v8i1.271
4. Nunan R, Harding KG, Martin P (2014) Clinical challenges of chronic wounds: searching for an optimal animal model to recapitulate their complexity. Dis Model Mech 7(11):1205–1213. https://doi.org/10.1242/dmm.016782
5. Leal EC, Carvalho E (2014) Cicatrização de Feridas: O fisiológico e O patológico wound healing: the physiologic and the pathologic. Revista Portuguesa De Diabetes 9:133–143
6. Martins AFM, Peres AA, Campos CS et al (2021) Perfil epidemiológico de lesões Cutâneas crônicas de Pacientes internados. Rev Enferm UFPE Line 15:e244519. https://doi.org/10.5205/1981-8963.2021.244519
7. Zhao R, Liang H, Clarke E et al (2016) Inflammation in chronic wounds. Int J Mol Sci 17(12):2085. https://doi.org/10.3390/ijms17122085
8. Cooper PO, Haas MR, Noonepalle SR, Shook BA (2021) Dermal drivers of Injury-Induced inflammation: contribution of adipocytes and fibroblasts. Int J Mol Sci 22(4):1933. https://doi.org/10.3390/ijms22041933
9. Harwood HJ (2012) The adipocyte as an endocrine organ in the regulation of metabolic homeostasis. Neuropharmacology 63(1):57–75. https://doi.org/10.1016/j.neuropharm.2011.12.010
10. Kovács D, Fazekas F, Oláh A, Törőcsik D (2020) Adipokines in the skin and in dermatological diseases. Int J Mol Sci 21(23):9048. https://doi.org/10.3390/ijms21239048
11. Miller NE, Michel CC, Nanjee MN et al (2011) Secretion of adipokines by human adipose tissue in vivo: partitioning between capillary and lymphatic transport. Am J Physiol Endocrinol Metab 301(4):E659–E667. https://doi.org/10.1152/ajpendo.00058.2011
12. Schmidt BA, Horsley V (2013) Intradermal adipocytes mediate fibroblast recruitment during skin wound healing. Development 140(7):1517–1527. https://doi.org/10.1242/dev.087593
13. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH (2001) Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 7(2):211–228. https://doi.org/10.1089/107632701300062859

14. Zou ML, Liu SY, Sun ZL et al (2020) Insights into the role of adipose-derived stem cells: wound healing and clinical regenerative potential. J Cell Physiol 236(4):2290–2297. https://doi.org/10.1002/jcp.30019
15. Medrado AR, Pugliese LS, Reis SR, Andrade ZA (2003) Influence of low level laser therapy on wound healing and its biological action upon myofibroblasts. Lasers Surg Med 32(3):239–244. https://doi.org/10.1002/lsm.10126
16. Bayer S, Kazancioglu HO, Acar AH et al (2017) Comparison of laser and Ozone treatments on oral mucositis in an experimental model. Lasers Med Sci 32:673–677. https://doi.org/10.1007/s10103-017-2166-1
17. Thomé Lima AMC, da Silva Sergio LP, da Silva Neto Trajano LA et al (2020) Photobiomodulation by dual-wavelength low-power laser effects on infected pressure ulcers. Lasers Med Sci 35:651–660. https://doi.org/10.1007/s10103-019-02862-w
18. Bavaresco T, Lucena AF (2022) Low-laser light therapy in venous ulcer healing: a randomized clinical trial. Revista Brasileira De Enfermagem 75(3):e20210396. https://doi.org/10.1590/0034-7167-2021-0396
19. KOUHKHEIL R, Fridoni M, Abdollhifar M et al (2019) Impact of photobiomodulation and condition medium on mast cell counts, degranulation, and wound strength in infected skin wound healing of diabetic rats. photobiomodulation, photomedicine, and laser surgery. 37(11):706–714. https://doi.org/10.1089/photob.2019.4691
20. Lima FQ, Marchionni AM, Medrado AP (2023) Histomorphometric evaluation of skin wounds in rats submitted to biomodulatory therapies– research protocol. Evid [Internet] 5:e4105. https://doi.org/10.17267/2675-021Xevidence.2023.e4105
21. Medrado AP, Costa T, Prado T et al (2010) Phenotype characterization of pericytes during tissue repair following low-level laser therapy. PhotoDermatol PhotoImmunol PhotoMed 26(4):192–197. https://doi.org/10.1111/j.1600-0781.2010.00521.x
22. Abreu JA, de Sousa C, Alves AdaL, Nunes CLGF JT (2011) Análise histológica Da Cicatrização de feridas Cutâneas Experimentais Sob Ação do laser de Baixa Potência. Sci Med [Internet] 21(3):96–100. https://revistaseletronicas.pucrs.br/ojs/index.php/scientiamedica/article/view/8223
23. Besser M, Schaeler L, Plattfaut I et al (2022) Pulsed low-intensity laser treatment stimulates wound healing without enhancing biofilm development in vitro. J Photochem Photobiol B 233:112504. https://doi.org/10.1016/j.jphotobiol.2022.112504
24. Lou Z, Gong T, Kang J, Photobiomodulation et al (2019) Photomed Laser Surg, 37(9):532–538. https://doi.org/10.1089/photob.2019.4641
25. de Oliveira LP (2019) Efeito do laser de Baixa Potência Em doses crescentes no processo de Cicatrização Cutânea Em Ratos. Dissertação, Universidade Federal de Goiás
26. Fonseca-Alaniz MH, Takada J, Alonso-Vale MIC, Lima FB (2006) O Tecido adiposo Como Centro regulador do metabolismo. Arq Bras Endocrinol Metab [Internet] 50(2):216–229. https://doi.org/10.1590/S0004-27302006000200008
27. Barthelemy J, Bogard G, Wolowczuk I (2023) Beyond energy balance regulation: the underestimated role of adipose tissues in host defense against pathogens. Front Immunol 14:1083191. https://doi.org/10.3389/fimmu.2023.1083191
28. Clemente-Suárez VJ, Redondo-Flórez L, Beltrán-Velasco AI et al (2023) The role of adipokines in health and disease. Biomedicines 11(5):1290. https://doi.org/10.3390/biomedicines11051290
29. Cunha MG, Cunha ALG, Machado CA (2014) Hipoderme e Tecido adiposo Subcutâneo: Duas estruturas diferentes. Surg Cosmet Dermatol 6(4):355–359
30. Driskell RR, Jahoda CA, Chuong CM, Watt FM, Horsley V (2014) Defining dermal adipose tissue. Exp Dermatol 23(9):629–631. https://doi.org/10.1111/exd.12450
31. Zhang Z, Shao M, Hepler C et al (2019) Dermal adipose tissue has high plasticity and undergoes reversible dedifferentiation in mice. J Clin Invest 129(12):5327–5342. https://doi.org/10.1172/JCI130239
32. Sastourné-Arrey Q, Mathieu M, Contreras X et al (2023) Adipose tissue is a source of regenerative cells that augment the repair of skeletal muscle after injury. Nat Commun 14:80. https://doi.org/10.1038/s41467-022-35524-7
33. Teng YY, Zou ML, Zhou XJ et al (2022) Novel prospects for scarless wound healing: the roles of myofibroblasts and adipocytes. J Cell Mol Med 26(20):5113–5121. https://doi.org/10.1111/jcmm.17535
34. Medrado AP et al (2008) Influence of laser photobiomodulation upon connective tissue remodeling during wound healing. J Photochem Photobiol B 92(3):144–152. https://doi.org/10.1016/j.jphotobiol.2008.05.008
35. Almadori A, Butler PE (2024) Scarring and skin fibrosis reversal with regenerative surgery and stem cell therapy. Cells 13(5):443. https://doi.org/10.3390/cells13050443
36. Marchionni AM, Medrado ARAP, Reis SRA (2007) Fotobiomodulação laser Em Tecido adiposo de Ratos. In: 24a. Reunião anual Da sociedade Brasileira de pesquisa odontológica, 2007, Atibaia. Brazilian oral research. São Paulo 21:209–209
37. Medrado ARAP, Pugliese LS, Reis SRA, Andrade Z (2001) A cronologia do Reparo Cutâneo - Influência do Raio laser de Baixa densidade de energia. In: 18A. Reunião anual Da sociedade Brasileira de pesquisa odontológica, Águas de Lindóia. Pesquisa Odontológica Brasileira 15:184–184
38. Shook BA, Wasko RR, Mano O et al (2020) Dermal adipocyte lipolysis and myofibroblast conversion are required for efficient skin repair. Cell Stem Cell 26(6):880–895e6. https://doi.org/10.1016/j.stem.2020.03.013
39. Pourhashemi E, Amini A, Ahmadi H et al (2024) Photobiomodulation and conditioned medium of adipose-derived stem cells for enhancing wound healing in rats with diabetes: an investigation on the proliferation phase. Lasers Med Sci 39:46. https://doi.org/10.1007/s10103-024-03974-8
40. Moravej FG, Amini A, Farahani RM et al (2023) Photobiomodulation, alone or combined with adipose-derived stem cells, reduces inflammation by modulation of microRNA-146a and interleukin-1ß in a delayed-healing infected wound in diabetic rats. Lasers Med Sci 38:129. https://doi.org/10.1007/s10103-023-03786-2
41. Kim YJ, Lee SH, Im J et al (2022) Increasing angiogenic efficacy of conditioned medium using light stimulation of human adipose-derived stem cells. Commun Biology 5(1):1–11. https://doi.org/10.1038/s42003-022-03838-3